Validation and application of a hormone assay for sea turtle sex ratio assessment

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Southwest and Northwest Fisheries Science Centers

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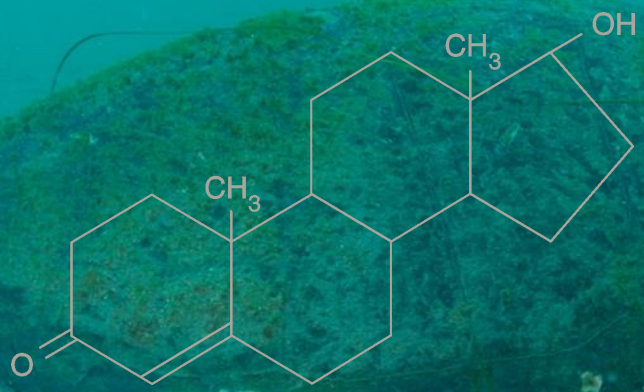
Sex Ratio data is important for status assessment modeling

Vital rate information is lacking for many sea turtle species



Important demographic information for status assessment modeling

Sex ratio is an important vital rate

Credit: Ryan Mable

Cannot use external morphology for sex determination of juvenile turtles

National and International Collaborations

ECKERD COLLEGE

Coonamessett Farm Foundation

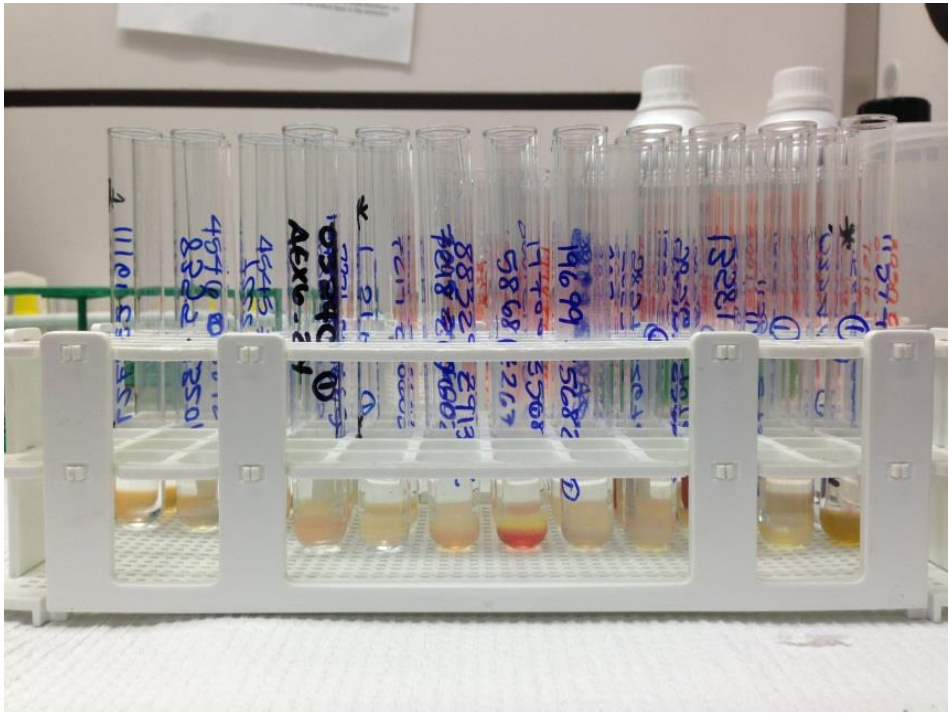
RIA vs. ELISA

- Radioimmunoassay (RIA)
  - Extremely sensitive (3.1 pg/mL)
  - Requires the use of radioactivity

- Enzyme-linked Immunosorbent Assay (ELISA)
  - As sensitive as RIA (2.0 pg/mL)
  - Do not require the use of radioactive substances

Methods

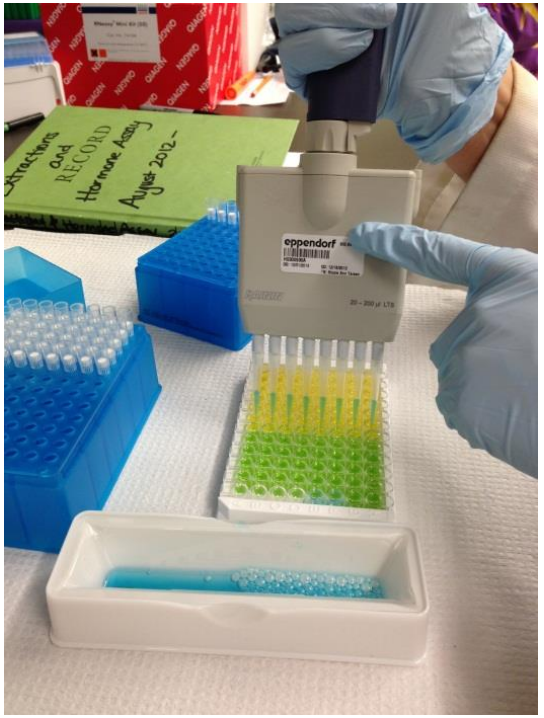
Extraction and isolation of hormones from plasma



Followed D. W. Owen's lab extraction methodology

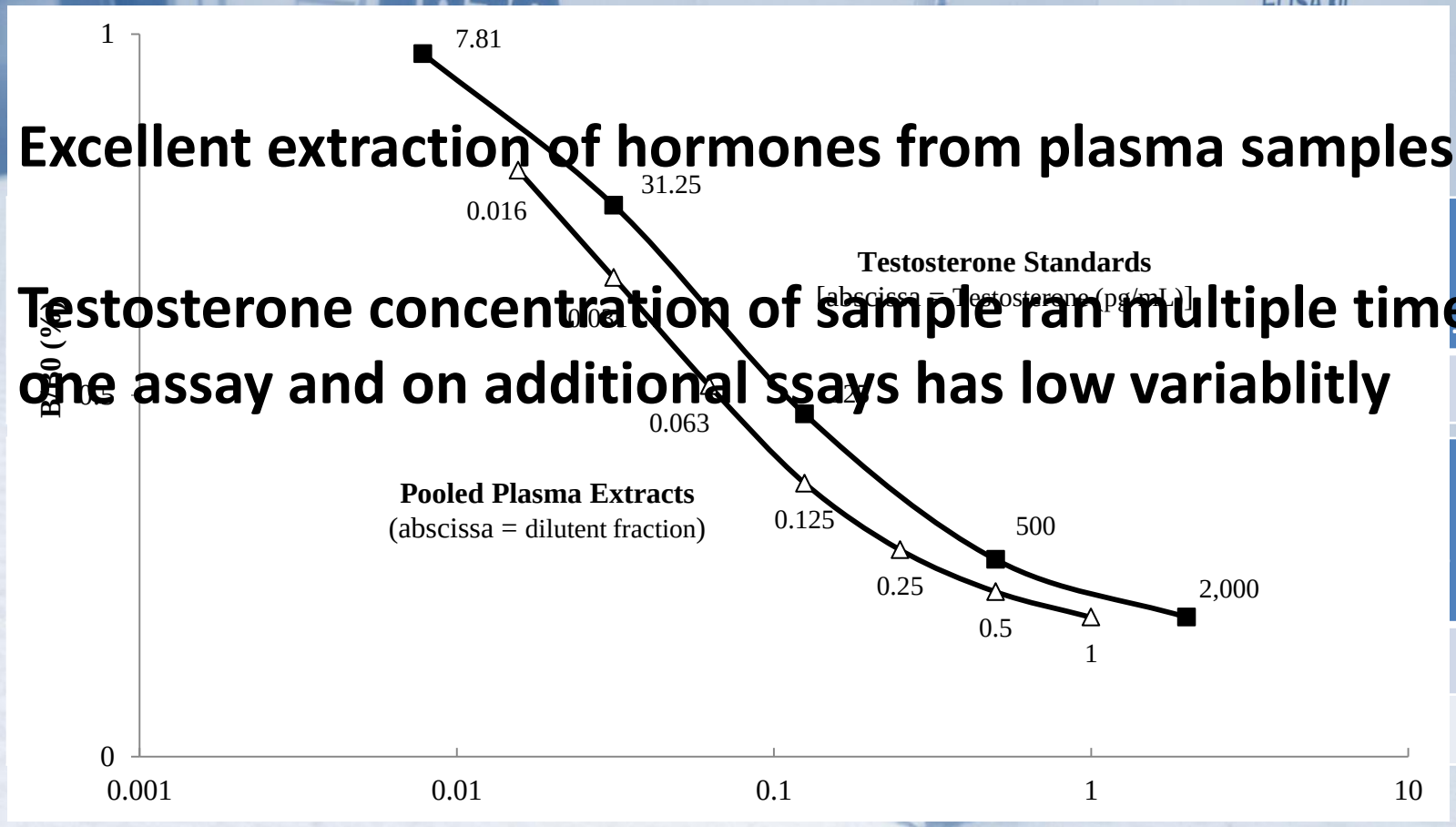
(Wibbles et al. 1987)

Quantify testosterone concentration via a colorimetric competitive enzyme immunoassay



Validated Testosterone ELISA for all 6 sea turtle species listed under the ESA

- Parallelism/linearity test demonstrated that the assay detects testosterone in plasma samples
- Excellent extraction of hormones from plasma samples
- Testosterone concentration of sample ran multiple times on one assay and on additional ssays has low variablitly

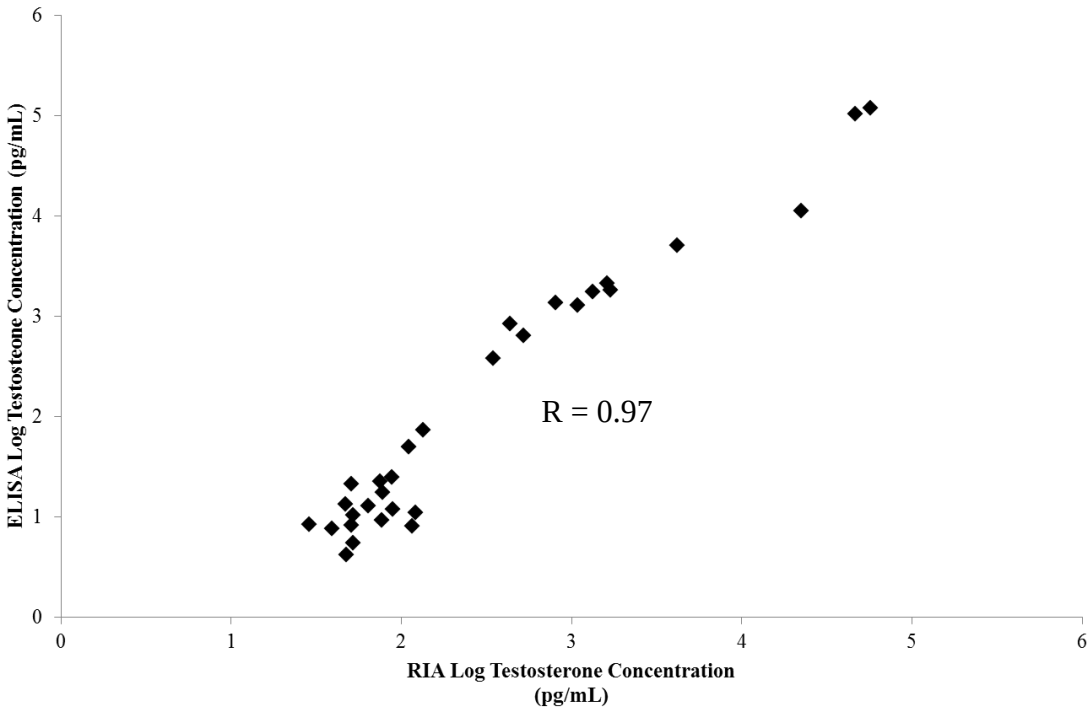


2003)

1992, 1993)

ELISA and RIA Comparison

Laparoscopy Sex	RIA Testosterone (pg/mL)	ELISA Testosterone (pg/mL)	ELISA Sex
Female	28.7	9.8	Female
Female	47.3	15.6	Female
Female	47.8	4.8	Female
Female	50.7	9.5	Female
Female	50.8	24.5	Female
Female	51.9	6.3	Female
Female	52.1	12.0	Female
Female	63.8	14.9	Female
Female	74.8	26.2	Female
Female	76.5	10.7	Female
Female	88.4	28.8	Female
Female	88.8	13.9	Female
Female	110.9	57.8	Female
Female	116.3	9.4	Female
Female	122.1	12.6	Female
Female	134.0	84.9	Female
Male	348.4	441.3	Male
Male	438.6	978.1	Male
Male	522.6	744.7	Male
Male	803.8	1,580	Male
Male	1,086	1,482	Male
Male	1,626	2,466	Male
Male	1,696	2,116	Male
Male	4,175	5,865	Male



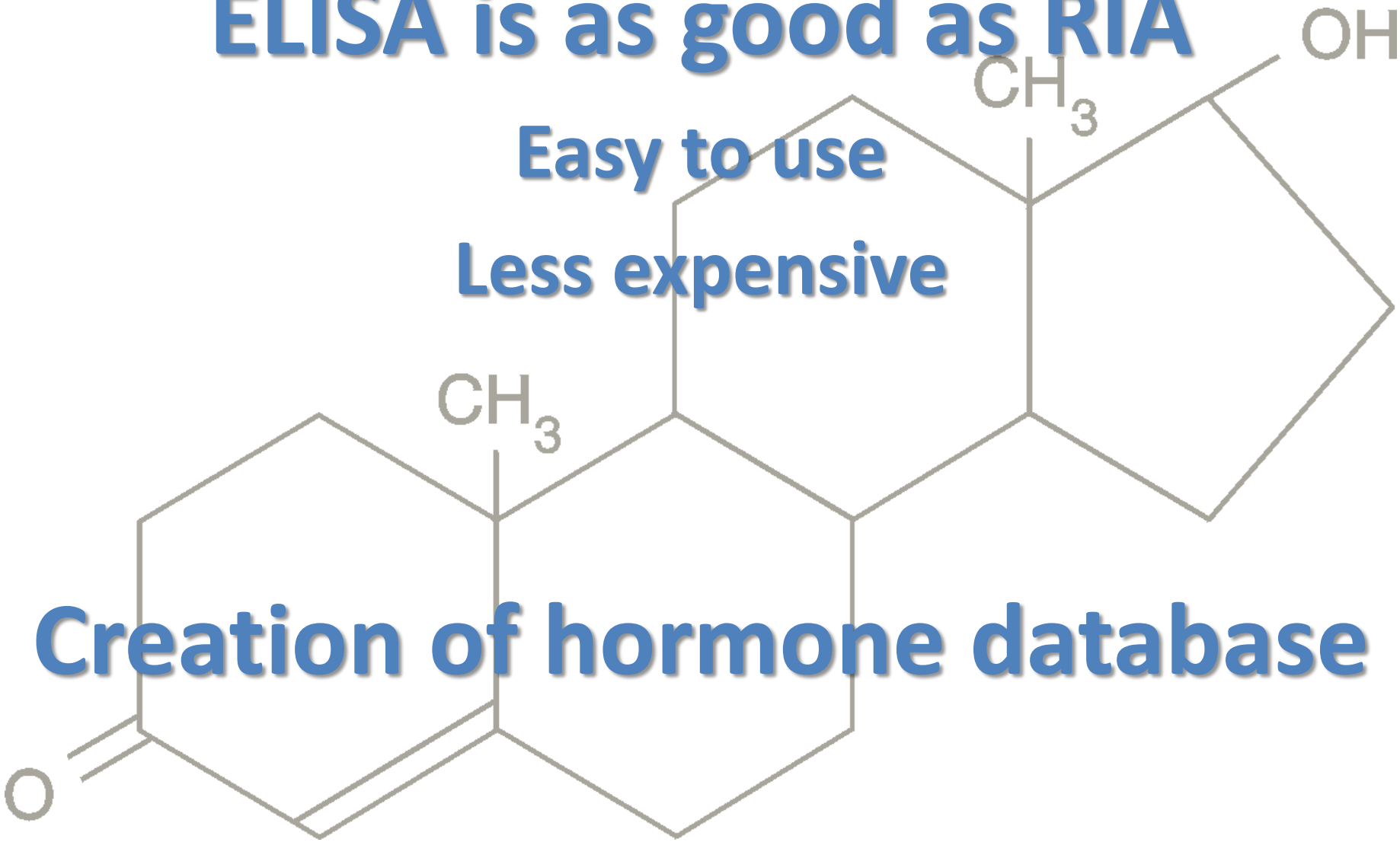
- Good correspondence between the two assays
- 100% for juvenile sex determination

Project Significance

ELISA is as good as RIA

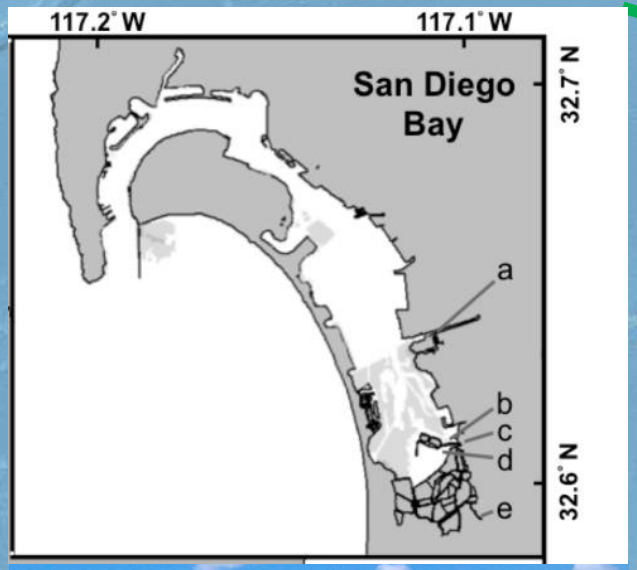
Easy to use

Less expensive



Creation of hormone database

Sample Collection Locations



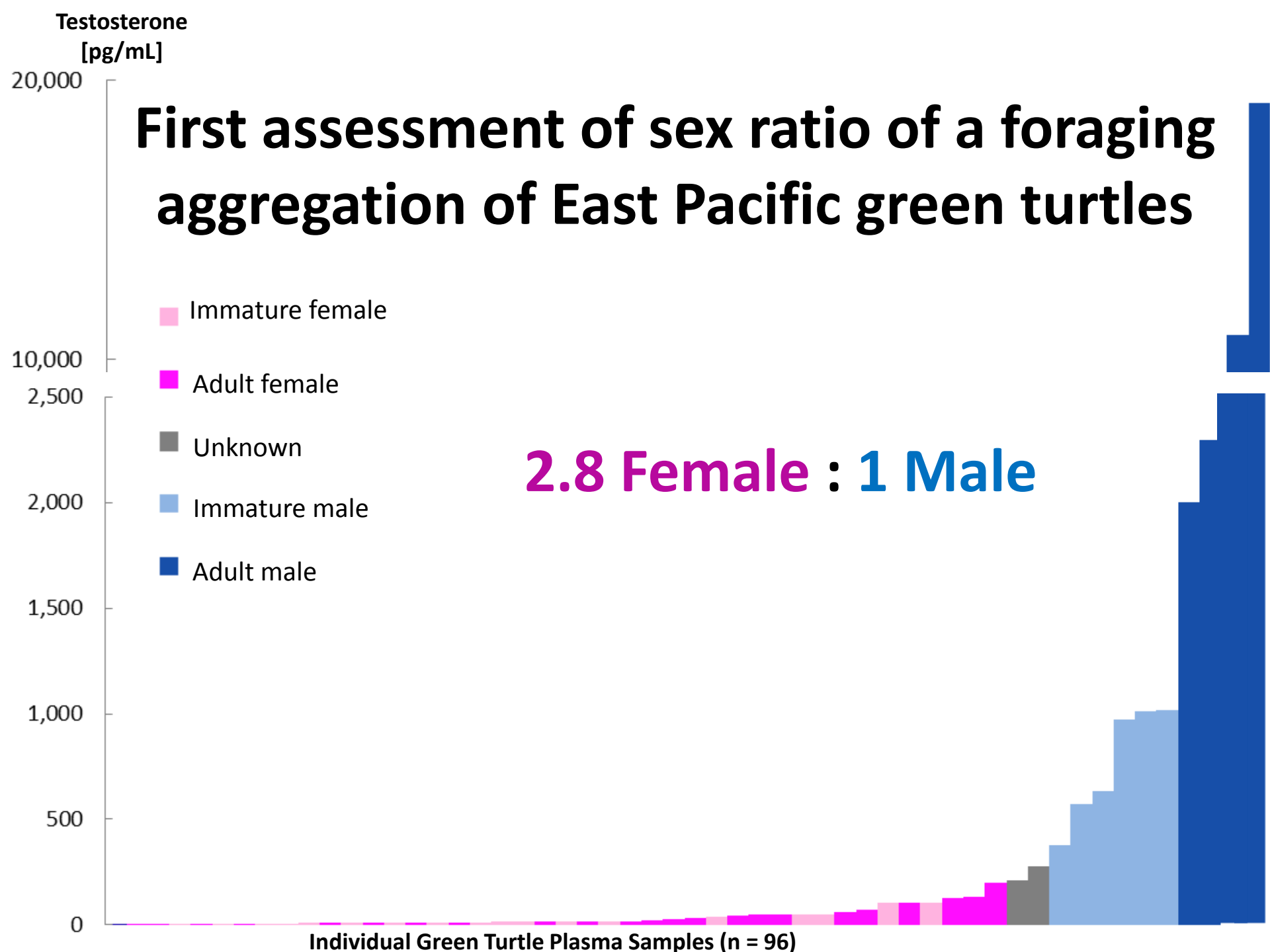
ETP STAR Cruise

Green	Kemp's Ridley
Leatherback	Olive Ridley
Loggerhead	Hawksbill

N

0 1,000 Km

Source: US National Park Service


Testosterone
[pg/mL]
20,000
10,000
2,500
2,000
1,500
1,000
500
0
First assessment of sex ratio of a foraging aggregation of East Pacific green turtles
Immature female
Adult female
Unknown
Immature male
Adult male
2.8 Female : 1 Male
Individual Green Turtle Plasma Samples (n = 96)

Comparison of sex ratio to other immature green turtle populations

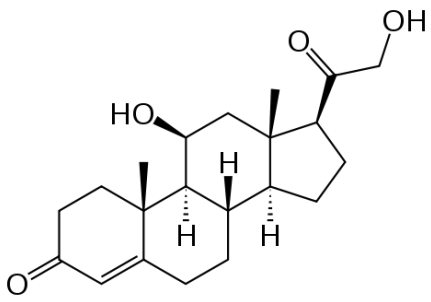
Immature Sex Ratio (F:M)	Location
0.96:1.0	Hawaii
1.40:1.0	Bahamas
2.0:1.0	Heron Island, Australia
3.26:1.0	Shoalwater Bay, Australia
3.5:1.0	**San Diego Bay, California**
4.0:1.0	Sabah, Malaysia
4.2:1.0	Clack Reef, Australia

Adapted from Owens (1997) in *The Biology of Sea Turtles*

Additional Hormone Projects

- Current
  - Hatchling sex ratios
  - Effects of temperature on testosterone
  - Placement of non-releasable turtles

- Future
  - Validate a corticosterone assay to examine stress response in sea turtles



Sea Turtle Endocrine Laboratory

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